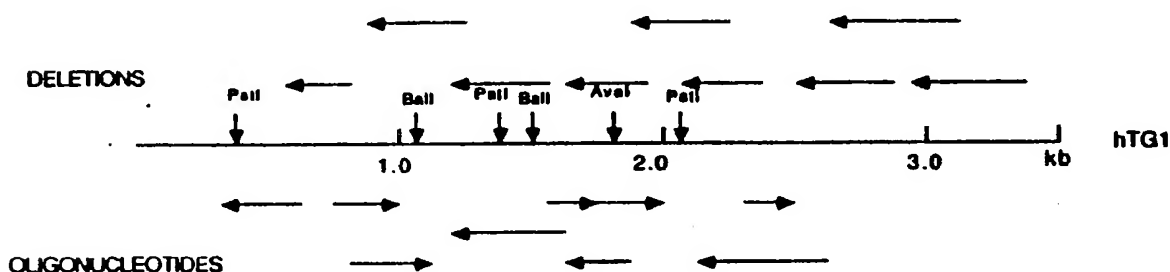




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(54) Title: CLONING AND EXPRESSION OF TISSUE TRANSGLUTAMINASES**(57) Abstract**

Human and murine tissue transglutaminases are cloned, sequenced and expressed. The tissue transglutaminases herein are useful for, *inter alia*, therapeutic wound repair, stabilizing food preparations, and markers for identifying agents which act as agonists or antagonists of cellular apoptosis.

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CLONING AND EXPRESSION OF TISSUE TRANSGLUTAMINASES

5

Background of the Invention

Transglutaminases are a group of calcium dependent
10 enzymes that catalyze the crosslinking of proteins by promoting
the formation of ϵ -(γ -glutaminyll)lysine isopeptide bonds
between protein-bound glutamine and lysine residues. These
enzymes are believed to be widely distributed in nature, as the
crosslinks are found in both prokaryotic and eukaryotic cells.
15 Although different transglutaminases appear to be very similar
in substrate specificity, several distinct forms of the enzymes
have been identified. See generally, Folk, Ann. Rev. Biochem.
49:517-531 (1980).

Transglutaminase-mediated protein crosslinking
20 reactions have been implicated in both normal and pathological
processes in mammalian cells and tissues. The crosslink may
act to maintain some forms of protein structure, such as in the
terminal differentiation of epidermal cell layers and in other
cellular architecture. An intracellular transglutaminase known
25 as epidermal or Type I transglutaminase has been isolated and
cloned from rabbit epithelial cells (Floyd and Jetten, Mol.
Cell. Biol. 9:4846-4851 (1989)), and a transglutaminase has
been isolated and cloned from guinea pig liver cells (Ikura et
al., Biochem. 27: 2898-2905 (1988)). Other transglutaminases
30 include hair follicle transglutaminase, keratinocyte
transglutaminase, and prostate transglutaminase (Wilson et al.,
Fed. Proc. 38:1809 (1979)). Lee et al., Prep. Biochem. 16:321-
335 (1986) have described the purification of a
transglutaminase from human erythrocytes. These
35 transglutaminases have been shown to be distinct from a plasma
transglutaminase, Factor XIII, an enzyme whose primary function
appears to be stabilizing fibrin clots. Factor XIII has also
been purified, cloned, and sequenced. (Ichinose, et al.,

Biochem. 25:6900-6906 (1986), Takahashi, et al., Proc. Natl. Acad. Sci. U.S.A. 83:8018-8023 (1986)).

5 The transglutaminases have been employed for crosslinking purposes in a variety of fields. Certain microbial transglutaminases have found use in food technology to add texture to processed foods, particularly fish and cheese. Others have been used in enzyme catalyzed fluorescent labeling of proteins, in the introduction of cleavable crosslinks, and in the solid phase reversible removal of
10 specific proteins from biological systems. Factor XIII preparations have been proposed for a variety of therapeutic uses, such as the treatment of subarachnoid hemorrhage and inflammatory bowel disease.

15 Presently, a plasma derived Factor XIII is available as a fibrin sealant, but, as with most plasma-derived products, carries an inherent risk of viral contamination. Further, Factor XIII and certain other transglutaminases are zymogens, requiring some form of activation to become catalytically active. And, as each transglutaminase has a restricted range
20 of substrates, their activity may be limited in certain applications. Accordingly, what is needed in the art are methods for producing by recombinant means human and murine transglutaminases, particularly those transglutaminases which do not require activation to become catalytically active. The
25 present invention fulfills these and other related needs.

Summary of the Invention

30 The present invention provides the ability to produce human and murine tissue transglutaminases and polypeptides or fragments thereof by recombinant means, preferably in cultured eukaryotic cells. The expressed transglutaminase may or may not have the biological activity of the native enzyme,
35 depending on the intended use. Accordingly, isolated and purified polynucleotides are described which code for the transglutaminases and fragments thereof, where the polynucleotides may be in the form of DNA, such as cDNA or

genomic DNA, or RNA. Based on these sequences probes may be designed for hybridization to identify these and related genes or transcription products thereof which encode human and murine tissue transglutaminases.

5 In related embodiments the invention concerns DNA constructs which comprise a transcriptional promoter, a DNA sequence which encodes the transglutaminase or fragment thereof, and a transcriptional terminator, each operably linked for expression of the enzyme or enzyme fragment. The
10 constructs are preferably used to transform or transfect host cells, preferably eukaryotic cells, more preferably yeast or mammalian cells. For large scale production the expressed transglutaminase may be isolated from the cells by, for example, immunoaffinity purification.

15 Nucleic acid sequences which encode the transglutaminases of the invention and the recombinant transglutaminases themselves can also be used to develop compounds which can alter transglutaminase-associated apoptosis of a eukaryotic cell. Compounds may be screened for agonistic
20 or antagonistic effects on transglutaminase-mediated metabolism in the host cell.

25 Brief Description of the Figures

Fig. 1 illustrates restriction maps of the mouse macrophage tissue transglutaminase cDNA clones, where the black portion of the boxes represents coding sequence for tissue transglutaminase, the white portion represents 3'-untranslated
30 sequences, and the restriction sites are represented as A=Alu I, B=BamH I, and N=Nco I;

Fig. 2 illustrates the sequencing strategy for the human cDNA insert in clone hTG1;

35 Fig. 3 illustrates nucleotide sequences of human endothelial (SEQ. ID. No. 1) and mouse macrophage (SEQ. ID. No. 3) tissue transglutaminases and their predicted amino acid sequences (SEQ. ID. NO. 2 and SEQ. ID. NO. 4, respectively), where the wavy lines indicate the amino acid sequence of the

pentapeptide containing the active site cysteine residue, the nucleotide sequence corresponding to a putative polyadenylation signal in the mouse sequence is located at the position 3452-3457, and the nucleotide sequence derived from mouse heart cDNA library has been underlined;

Fig. 4 illustrates the identification of human tissue transglutaminase mRNA by blot hybridization, analyzing 10 µg of mRNA from HUVEC; and

Fig. 5 illustrates the identification of mouse transglutaminase mRNA by blot hybridization, analyzing 10 µg of mRNA from each of the following tissues: L, liver; S, spleen; K, kidney; T, testis; H, heart; Lu, lung; Tm, thymus; and B, brain.

Description of the Preferred Embodiments

Tissue transglutaminase (or transglutaminase II) is an enzyme that catalyzes the crosslinking of protein-bound glutamine and primary amines, such as lysine residues. The present invention provides isolated nucleotide sequences of human tissue transglutaminase, thereby providing for the ultimate expression of human tissue transglutaminase polypeptides. Recombinant DNA expression systems provide convenient means for obtaining large quantities of human tissue transglutaminases in relatively pure form. The invention also provides cloned nucleotide sequences of murine tissue transglutaminase.

The invention also provides recombinant human and murine tissue transglutaminase polypeptides and fragments thereof having transglutaminase activity. By polypeptides and fragments is meant to include sequences of amino acids up to entire proteins, which have at least about 85% homology, preferably at least 90%, and more preferably at least about 95% or more homology to the amino acid sequences of the murine or human sequences of the invention, as shown in Fig. 3 and SEQ. ID. Nos. 1-4. As will be appreciated by those skilled in the art, the invention also includes those polypeptides having

slight variations in amino acid sequences or other properties. Such variations may arise naturally as allelic variations (e.g., due to genetic polymorphism) or may be produced by human intervention (e.g., by mutagenesis of cloned DNA sequences),
5 such as induced point, deletion and insertion mutants.

Nucleic acid sequences encoding human tissue transglutaminase as described herein can be cloned from a variety of human cell sources that express the enzyme. Preferred sources include human umbilical vein endothelial
10 cells and retinoic acid stimulated macrophages. Useful nucleic acid sequences in this regard include mRNA, genomic DNA and cDNA. For expression, cDNAs are generally preferred because they lack introns that may interfere with expression. To obtain a human tissue transglutaminase clone, a human
15 endothelial cell cDNA library is screened with, e.g., labeled probes from random primed mouse macrophage transglutaminase sequences, which probes preferably span the enzyme's active site and/or putative calcium binding site. To obtain the mouse tissue transglutaminase clone, an oligo-dT primed cDNA library
20 can be constructed with polyA⁺ RNA purified from mouse peritoneal macrophages stimulated with retinoic acid. The library is screened with, e.g., polyclonal antibodies to guinea pig liver tissue transglutaminase and/or labeled RNA probes. Partial clones may be used as probes in additional screening
25 until the complete coding sequence is obtained. If necessary, partial clones are joined in the correct reading frame to construct the complete coding sequence. Joining is achieved by digesting clones with appropriate restriction endonucleases and joining the fragments enzymatically in the proper orientation.
30 Depending on the fragments and the particular restriction endonucleases chosen, it may be necessary to remove unwanted DNA sequences through a "loop out" process of deletion mutagenesis or through a combination of restriction endonuclease cleavage and mutagenesis. It is preferred that
35 the resultant sequence be in the form of a continuous open reading frame, that is, that it lack intervening sequences (introns). The sequence of one exemplary mouse clone described herein, TGHZ3, includes 29 nucleotides of 5'-untranslated

sequence and 1,775 nucleotides of coding sequence and is shown in Fig. 3 (SEQ. ID. NO. 3).

5 A human cDNA transglutaminase clone isolated as described herein includes the entire 5'-untranslated sequence, as determined by primer extension analysis, the coding domain, and 1,058 nucleotides of 3'-untranslated sequence, as shown in Fig. 3 (SEQ. ID. NO. 1). This clone lacks a consensus polyadenylation sequence and is slightly shorter than the 3.6 kb full length transcript, as determined by Northern blot
10 analysis of human endothelial cell RNA, suggesting that it lacks approximately 300 bp of 3'-untranslated sequence. The identity of the human tissue transglutaminase clone is confirmed by, for example, in vitro translation. As described further below, clone hTG-1 encodes a polypeptide that migrates
15 at Mr 80,000 on SDS-polyacrylamide gels. Its deduced molecular weight is 77,253. The active site Cys residue was determined to be at position 277 as shown in Fig. 3.

With the nucleotide and deduced amino acid sequences of human tissue transglutaminase provided herein, genomic or
20 cDNA sequences encoding tissue transglutaminase may be obtained from libraries prepared from other cells and tissues according to known procedures. For instance, using oligonucleotide probes derived from human endothelial transglutaminase sequences, generally of at least about fourteen nucleotides and
25 up to twenty-five or more nucleotides in length, DNA sequences encoding transglutaminase of other tissues and/or mammalian species may be obtained. If partial clones are obtained, it is necessary to join them in proper reading frame to produce a full length clone, using such techniques as endonuclease
30 cleavage, ligation and loopout mutagenesis.

For expression, a DNA sequence encoding tissue transglutaminase is inserted into a suitable expression vector, which in turn is used to transform or transfect appropriate host cells for expression. Expression vectors for use in
35 carrying out the present invention will comprise a promoter capable of directing the transcription of a cloned DNA and a transcriptional terminator, operably linked with the sequence encoding the tissue transglutaminase so as to produce a

continuously transcribable gene sequence which produces sequences in reading frame and continuously translated to produce a transglutaminase polypeptide.

Host cells for use in practicing the present invention include mammalian, avian, plant, insect, bacterial and fungal cells, but preferably eukaryotic cells. Preferred eukaryotic cells include cultured mammalian cell lines (e.g., rodent or human cell lines) and fungal cells, including species of yeast (e.g., Saccharomyces spp., particularly S. cerevisiae, Schizosaccharomyces spp., or Kluyveromyces spp.) or filamentous fungi (e.g., Aspergillus spp., Neurospora spp.). Methods for producing recombinant proteins in a variety of prokaryotic and eukaryotic host cells are generally known in the art.

Techniques for transforming fungi are well known in the literature, and have been described, for instance, by Beggs (ibid.), Hinnen et al. (Proc. Natl. Acad. Sci. USA 75: 1929-1933, 1978), Yelton et al. (Proc. Natl. Acad. Sci. USA 81: 1740-1747, 1984), and Russell (Nature 301: 167-169, 1983). The genotype of the host cell will generally contain a genetic defect that is complemented by the selectable marker present on the expression vector. Choice of a particular host and selectable marker is well within the level of ordinary skill in the art.

Suitable yeast vectors for use in the present invention include YRp7 (Struhl et al., Proc. Natl. Acad. Sci. USA 76: 1035-1039, 1978), YEpl3 (Broach et al., Gene 8: 121-133, 1979), POT vectors (Kawasaki et al, U.S. Patent No. 4,931,373, which is incorporated by reference herein), pJDB249 and pJDB219 (Beggs, Nature 275:104-108, 1978) and derivatives thereof. Such vectors will generally include a selectable marker, which may be one of any number of genes that exhibit a dominant phenotype for which a phenotypic assay exists to enable transformants to be selected. Preferred selectable markers are those that complement host cell auxotrophy, provide antibiotic resistance or enable a cell to utilize specific carbon sources, and include LEU2 (Broach et al., ibid.), URA3 (Botstein et al., Gene 8: 17, 1979), HIS3 (Struhl et al., ibid.) or POT1 (Kawasaki et al., ibid.). Another suitable

selectable marker is the CAT gene, which confers chloramphenicol resistance on yeast cells.

Preferred promoters for use in yeast include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255: 12073-12080, 1980; Alber and Kawasaki, J. Mol. Appl. Genet. 1: 419-434, 1982; Kawasaki, U.S. Patent No. 4,599,311) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals, Hollaender et al., (eds.), p. 355, Plenum, New York, 1982; Ammerer, Meth. Enzymol. 101: 192-201, 1983). In this regard, particularly preferred promoters are the TPI1 promoter (Kawasaki, U.S. Patent No. 4,599,311, 1986) and the ADH2-4^C promoter (Russell et al., Nature 304: 652-654, 1983; Irani and Kilgore, U.S. Patent Application Serial No. 183,130, which is incorporated herein by reference). The expression units may also include a transcriptional terminator. A preferred transcriptional terminator is the TPI1 terminator (Alber and Kawasaki, *ibid.*).

Additional vectors, promoters and terminators for use in expressing the transglutaminases of the invention in yeast are well known in the art and are reviewed by, for example, Emr, Meth. Enzymol. 185:231-279, (1990), incorporated herein by reference.

The transglutaminases of the invention may be expressed in Aspergillus spp. (McKnight and Upshall, described in U.S. Patent 4,935,349, which is incorporated herein by reference). Useful promoters include those derived from Aspergillus nidulans glycolytic genes, such as the ADH3 promoter (McKnight et al., EMBO J. 4:2093-2099, 1985) and the tpiA promoter. An example of a suitable terminator is the ADH3 terminator (McKnight et al., *ibid.*). Techniques for transforming fungi are well known in the literature, and have been described, for instance by Beggs (*ibid.*), Hinnen et al. (Proc. Natl. Acad. Sci. USA 75:1929-1933, 1978), Yelton et al. (Proc. Natl. Acad. Sci. USA 81:1740-1747, 1984), and Russell (Nature 301:167-169, 1983) each of which are incorporated herein by reference.

In addition to fungal cells, cultured mammalian cells may be used as host cells within the present invention.

Preferred cultured mammalian cells for use in the present invention include the COS-1 (ATCC CRL 1650) and BALB/c 3T3 (ATCC CRL 163) cell lines. In addition, a number of other mammalian cell lines may be used within the present invention, including BHK (ATCC CRL 10314), 293 (ATCC CRL 1573), Rat Hep I (ATCC CRL 1600), Rat Hep II (ATCC CRL 1548), TCMK (ATCC CCL 139), Human lung (ATCC CCL 75.1), Human hepatoma (ATCC HTB-52), Hep G2 (ATCC HB 8065), Mouse liver (ATCC CCL 29.1), NCTC 1469 (ATCC CCL 9.1) and DUKX cells (Urlaub and Chasin, Proc. Natl. Acad. Sci USA 77: 4216-4220, 1980).

Mammalian expression vectors for use in carrying out the present invention will include a promoter capable of directing the transcription of a cloned gene or cDNA. Preferred promoters include viral promoters and cellular promoters. Viral promoters include the immediate early cytomegalovirus promoter (Boshart et al., Cell 41: 521-530, 1985), the SV40 promoter (Subramani et al., Mol. Cell. Biol. 1: 854-864, 1981), and the major late promoter from Adenovirus 2 (Kaufman and Sharp, Mol. Cell. Biol. 2: 1304-1319, 1982). Cellular promoters include the mouse metallothionein-1 promoter (Palmiter et al., U.S. Patent No. 4,579,821), a mouse V_{κ} promoter (Bergman et al., Proc. Natl. Acad. Sci. USA 81: 7041-7045, 1983; Grant et al., Nuc. Acids Res. 15: 5496, 1987) and a mouse V_H promoter (Loh et al., Cell 33: 85-93, 1983). Also contained in the expression vectors is a polyadenylation signal located downstream of the coding sequence of interest. Polyadenylation signals include the early or late polyadenylation signals from SV40 (Kaufman and Sharp, *ibid.*), the polyadenylation signal from the Adenovirus 5 E1B region and the human growth hormone gene terminator (DeNoto et al., Nuc. Acids Res. 9: 3719-3730, 1981). Vectors can also include enhancer sequences, such as the SV40 enhancer and the mouse μ enhancer (Gillies, Cell 33: 717-728, 1983). Expression vectors may also include sequences encoding the adenovirus VA RNAs. Vectors can be obtained from commercial sources (e.g., Stratagene, La Jolla, CA).

Cloned DNA sequences may be introduced into cultured mammalian cells by, for example, calcium phosphate-mediated

transfection (Wigler et al., Cell 14: 725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7: 603, 1981; Graham and Van der Eb, Virology 52: 456, 1973), electroporation (Neumann et al., EMBO J. 1: 841-845, 1982), or DEAE-dextran mediated
5 transfection (Ausubel et al., (ed.) Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY (1987), incorporated herein by reference). To identify cells that have stably integrated the cloned DNA, a selectable marker is generally introduced into the cells along with the gene or cDNA
10 of interest. Preferred selectable markers for use in cultured mammalian cells include genes that confer resistance to drugs, such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. Preferred amplifiable selectable markers are the DHFR gene and the
15 neomycin resistance gene. Selectable markers are reviewed by Thilly (Mammalian Cell Technology, Butterworth Publishers, Stoneham, MA, which is incorporated herein by reference). The choice of selectable markers is well within the level of ordinary skill in the art.

20 Selectable markers may be introduced into the cell on a separate vector at the same time as the transglutaminase sequence of interest, or they may be introduced on the same vector. If on the same vector, the selectable marker and the transglutaminase sequence of interest may be under the control
25 of different promoters or the same promoter, the latter arrangement producing a dicistronic message. Constructs of this type are known in the art (for example, Levinson and Simonsen, U.S. Patent No. 4,713,339). It may also be advantageous to add additional DNA, known as "carrier DNA" to
30 the mixture which is introduced into the cells.

Transfected mammalian cells are allowed to grow for a period of time, typically 1-2 days, to begin expressing the DNA sequence(s) of interest. Drug selection is then applied to select for growth of cells that are expressing the selectable
35 marker in a stable fashion. For cells that have been transfected with an amplifiable selectable marker the drug concentration may be increased in a stepwise manner to select for increased copy number of the cloned sequences, thereby

increasing expression levels.

Promoters, terminators and methods for introducing expression vectors encoding transglutaminase into plant, avian and insect cells are well known in the art. The use of baculoviruses, for example, as vectors for expressing heterologous DNA sequences in insect cells has been reviewed by Atkinson et al. (Pestic. Sci. 28: 215-224, 1990). The use of Agrobacterium rhizogenes as vectors for expressing genes in plant cells has been reviewed by Sinkar et al. (J. Biosci. (Bangalore) 11: 47-58, 1987).

Host cells containing DNA constructs of the present invention are then cultured to produce the transglutaminase. The cells are cultured according to standard methods in a culture medium containing nutrients required for growth of the chosen host cells. A variety of suitable media are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals, as well as other components, e.g., growth factors or serum, that may be required by the particular host cells. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker on the DNA construct or co-transfected with the DNA construct.

Yeast cells, for example, are preferably cultured in a medium which comprises a nitrogen source (e.g., yeast extract), inorganic salts, vitamins and trace elements. The pH of the medium is preferably maintained at a pH greater than 2 and less than 8, preferably at pH 5-6. Methods for maintaining a stable pH include buffering and constant pH control, preferably through the addition of sodium hydroxide. Preferred buffering agents include succinic acid and Bis-Tris (Sigma Chemical Co., St. Louis, MO). Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media. Selection of a medium appropriate for the particular cell line used is within the level of ordinary skill in the art.

In a preferred embodiment, human tissue transglutaminase is expressed in yeast as an intracellular

product. The yeast host is a diploid strain homozygous for pep4, a mutation that reduces vacuolar protease levels, as described in Jones et al., Genetics 85:23-33 (1977), incorporated herein by reference. The strain is also

5 homozygous for disruption of the endogenous TPI (triose phosphate isomerase) gene, thereby allowing the S. pombe POT1 gene to be used as a selectable marker. The vector includes the POT1 marker, a leu2-d marker and the ADH2-4c promoter. The POT1 marker in the TPI⁻ host allows for selection by growth in

10 glucose. The host strain is grown in glucose-containing synthetic media with a glucose feed. An ethanol feed is then substituted for glucose to de-repress the promoter. The pH is maintained with NaOH. Other preferred means for expression are generally described in, e.g., EPO publication EP 268,772,

15 incorporated herein by reference.

The tissue transglutaminase produced according to the present invention may be purified by affinity chromatography on an antibody column using antibodies directed against

20 transglutaminase. Additional purification may be achieved by conventional chemical purification means, such as liquid chromatography, gradient centrifugation, and gel electrophoresis, among others. Methods of protein purification are known in the art (see generally, Scopes, R., Protein Purification, Springer-Verlag, NY (1982), which is incorporated

25 herein by reference) and may be applied to the purification of the recombinant transglutaminase described herein. Substantially pure recombinant tissue transglutaminase of at least about 50% is preferred, at least about 70-80% more preferred, and 95-99% or more homogeneity most preferred,

30 particularly for pharmaceutical uses. Once purified, partially or to homogeneity, as desired, the recombinant tissue transglutaminase may then be used in food preparation, protein chemistry, therapeutically, etc.

The human and murine tissue transglutaminases

35 produced according to the present invention find a variety of uses. For example, tissue transglutaminases may be used in the preparation of food material, such as paste food, cheese, and can be added to dehydrated fish to prevent deterioration caused

by protozoans, e.g., myxamoeba. The transglutaminases can also be used in the preparation of ground meat of okiomi (Euphasia superba), by adding to dehydrated meat parts from 0.1 to 100 units, preferably about 1-40 U per gram of protein to improve meat texture and quality. Frozen granular meats can be improved by combining meat material with tissue transglutaminase of the invention at 1-500 U per gram protein, at 30-60°C for 10-120 min. to promote crosslinking between glutamine groups and lysine contained in meat preparations.

Other uses of the tissue transglutaminases described herein include the enzyme-catalyzed labeling of proteins and cell membranes (Iwanij, Eur. J. Biochem. 80:359-368 (1977), incorporated herein by reference), in the introduction of cleavable crosslinks, and in the solid phase reversible removal of specific proteins from biological systems.

The human transglutaminase of the invention also can be used therapeutically in humans. For example, the transglutaminase may be used in the repair of wounds and ulcerated lesions. As the tissue enzyme is relatively stable, active extracellularly, and binds avidly to collagen, it can be used to stabilize basement membrane structures. An appropriate endogenous substrate for the enzyme is fibronectin, which thus serves as a basis for crosslinking and stabilizing collagen/fibronectin complexes.

Transglutaminase expression can be used as a marker for screening for agonists and antagonists of cellular apoptosis. Identifying agents which inhibit the expression of transglutaminase by a cell provides a means to prevent or delay atrophic changes characteristic of many degenerative changes, particularly degenerative nerve diseases, such as Parkinson's disease and Alzheimer's disease. Inhibition of apoptosis may also enhance blood cell counts in chemotherapy patients. The tissue transglutaminase or the nucleic acids which encode the tissue transglutaminase of the invention can also be used to identify agents which induce apoptotic activity by a cell, for the control of, e.g., hyperproliferative disorders. The growth of cells such as adipocytes can be regulated with agents identified using the tissue transglutaminases provided herein

as a marker, providing a means for controlling fat depots in certain forms of obesity without the necessity for surgical intervention.

Sequences which encodes transglutaminases may be directly detected in cells with labeled synthetic oligonucleotide probes in a hybridization procedure similar to the Southern or dot blot. Also, the polymerase chain reaction (Saiki et al., Science 239:487 (1988), and U.S. Pat. No. 4,683,195) may be used to amplify DNA sequences, which are subsequently detected by their characteristic size on agarose gels, Southern blot of the gels using transglutaminase sequences or a oligonucleotide probe, or a dot blot using similar probes. The probes may comprise from about 14 nucleotides to about 25 or more nucleotides, sometimes 40 to 60 nucleotides, and in some instances a substantial portion or even the entire cDNA of a transglutaminase gene of the invention may be used. The probes are labeled with a detectable signal, such as an enzyme, biotin, a radionuclide, fluorophore, chemiluminescer, paramagnetic particle, etc.

The following examples are provided by way of illustration, not limitation.

EXAMPLE I

Cloning of Mouse and Human Tissue Transglutaminases

The following example describes the cDNA cloning and screening of mouse macrophage and heart tissue transglutaminase and human endothelial cell transglutaminase.

An oligo-dT primed cDNA library was constructed in the lambda gt11 vector with polyA⁺ RNA purified from mouse peritoneal macrophages essentially as described by Chiocca et al., J. Biol. Chem. 263:11584-11589 (1988), incorporated herein by reference. Briefly, mice (1200) were sacrificed and their peritoneal cavities were washed with RPMI. Cells were harvested from the wash by centrifugation. The cells were plated and macrophages were allowed to attach to the dishes for 60 minutes. The dishes were then washed and the macrophages

were recovered. To induce transglutaminase expression, the macrophages were stimulated with retinoic acid (10^{-6} M) for 6 hours. RNA was then isolated from the cells, cDNA was synthesized and *E. coli* cells were infected with recombinant phages.

To screen the recombinant clones goat polyclonal antibodies (made against guinea pig liver transglutaminase, as described in Murtagh et al., *J. Biol. Chem.* 258:11074-11081 (1983), incorporated herein by reference) were used. Clones TG700 and TG1600 were identified, and [32 P]-labeled RNA transcripts from these clones were used to rescreen the library, as generally described in Ausubel et al., *supra*. Two positive clones identified in the second screening were designated TG3000 and TG3400. Since none of the clones isolated in these initial rounds of screening was a full length sequence or encoded the 5'-end of the cDNA, the macrophage library was then subjected to two more rounds of screening with short hybridization probes prepared from the 5'-ends of TG3000 and TG3400 (solid bars Fig. 1). All of these clones stopped short of the translation start site, so a mouse heart cDNA library cloned into lambda ZAP (Stratagene) was screened with an oligonucleotide hybridization probe derived from the 5'-end of clone TG7.4 (Fig. 1). A heart transglutaminase cDNA isolated by this procedure (clone TGHZ3) included 29 nucleotides of 5'-untranslated region, the initiation codon ATG and 1775 nucleotides of coding sequence.

Primer extension analysis of RNA derived from control and retinoic acid stimulated mouse macrophages, performed as generally described in Ausubel et al., *ibid.*, and Maniatis et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1982), incorporated by reference herein, was used to locate the transcription start site. A synthetic oligonucleotide of sequences included in the 5' end of cDNA clone TG7.4 was hybridized to macrophage RNA and then transcripts were synthesized in the presence of 32 P-dATP. A single prominent 255 nucleotide band, more abundant in transcripts from retinoic acid-stimulated than control macrophage RNA was detected, locating the transcription start

site at a position 103 nucleotides upstream of the initiation ATG.

Repetitive screening of a mouse macrophage and heart cDNA libraries resulted in the isolation of 12 overlapping cDNA clones encoding 3487 nucleotides that spanned nearly the entire mouse tissue transglutaminase mRNA. The overlapping clones included a consensus polyadenylation signal at the 3'-end. The 5'-end of the clone isolated from mouse heart library was 75 nucleotides short of the transcript start site. The clones were sequenced as described in Example II below.

To clone the human endothelial cell transglutaminase, an oligo-dT and random-primed cDNA library was constructed with polyA⁺ RNA from human umbilical vein endothelial cells (HUVEC) in the vector lambda ZAP (Stratagene Inc., La Jolla, CA). X-LI blue cells (Stratagene) were infected with recombinant phages and 2 x 10⁵ plaques were screened with a random-primed mouse macrophage transglutaminase [³²P]-labeled DNA probe spanning the active site and the putative calcium binding domain (TG7.4) to facilitate isolation of full length cDNA. The hybridization procedure was done generally as described in Ausubel et al., *ibid.*, at 55°C overnight with a final wash at 60°C in 0.1% SSC/0.1% SDS for 30 minutes. The initial screen of 2 x 10⁵ recombinant phage yielded 5 positive clones. The inserts in three of the clones, hTG2, 3 and 5, were totally included within the largest cDNA clone, hTG1, which was approximately 3.3 kilobases.

To locate the transcription start site of human endothelial transglutaminase mRNA, a primer complementary to nucleotide positions 53 to 72 was synthesized and used to determine the size of the cDNA extension product. This reaction resulted in a transcript of 52 nucleotides, indicating the transcription start site is 135 nucleotides upstream from the initiator ATG and is likely coincident with the 5'-end of clone hTG-1.

EXAMPLE II

Nucleotide Sequence Analysis of Human and
Mouse Tissue Transglutaminase cDNA Clones

5 The following Example describes the sequencing of mouse and human cDNA clones obtained in Example I. The results show a substantial degree of sequence homology between the two species of tissue enzyme.

10 To determine the human tissue transglutaminase sequence, both strands of the human tissue transglutaminase cDNA clone (hTG-1) were sequenced by the dideoxy chain termination method with a Sequenase enzyme kit using synthetic oligonucleotide primers and deleted clones derived by
15 exonuclease digestion (Fig. 2). As shown in Fig. 3 (SEQ. ID. NO. 1), the 3257 nucleotides included a single open reading frame encoding 687 amino acids (also SEQ.ID. NO. 2). An initiation codon, located 136-138 nucleotides downstream from the transcription start site, was included within a consensus sequence (ACCATGG) recognized as optimal for the initiation of
20 eukaryotic translation (Kozak sequence; Kozak, Cell 44:283-292 (1986)). A terminator codon (TAA), located at nucleotide 2194-2196, was followed by 1058 nucleotides of 3'-untranslated sequence. No consensus polyadenylation signal sequence was recognized in the 3'-untranslated region.

25 The nucleotide sequence of the mouse tissue transglutaminase (Fig. 3) (SEQ. ID. No. 3) was determined by sequencing of overlapping cDNA clones (Fig. 1). CsCl purified, double-stranded mouse cDNA was sequenced by both the chemical degradation (Maxam and Gilbert, Proc. Natl. Acad. Sci. USA
30 74:560-564 (1977)) and the dideoxy chain termination (Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977)) methods.

35 The entire murine transcription unit sequence was slightly larger than the human transglutaminase cDNA clone (Fig. 3). An open reading frame of 2055 nucleotides encoded a protein of 685 amino acid residues (SEQ. ID. NO. 4). The initiation ATG was included in a Kozak sequence. The terminator triplet (TAA) was followed by 1400 bp of 3'-untranslated sequence that included a consensus polyadenylation

sequence (AATAAA) at the 3' end of the clone.

The DNA and amino acid sequences were analyzed with computer programs NUCALN and PRTALN. See Wilbur and Lipman, Proc. Natl. Acad. Sci. USA 80:726-730 (1983), incorporated herein by reference. Fig. 3 compares the nucleotide sequences of the human and mouse tissue transglutaminase cDNA's. The sequence of the human enzyme is fully represented in the top line and the deduced amino acid sequence of the coding domain is shown below. The third line contains the deduced amino acid sequence of the mouse tissue transglutaminase. Residues identical with the human enzyme are shown in an asterisk, residues that are distinct are shown with the single letter code. The fourth line shows the nucleotide sequence of the mouse transglutaminase. Nucleotides identical to the human enzyme are shown with a dash.

Comparison of the overall nucleotide sequence of the human and mouse coding domains shows a high degree (>82%) of homology. Most substitutions are silent mutations in the third position of codons. The 3' untranslated region of the two cDNA's showed no significant homology. The overall homology at the amino acid level was 84%. Table I compares the amino acid composition and the calculated molecular weight of human and mouse tissue transglutaminase.

TABLE I: Amino acid composition of human and mouse tissue transglutaminases.

	Human Endothelial		Mouse Macrophage
	amino acid	n. res./mol.	
	F =	25	22
15	L =	69	70
	I =	32	30
	M =	11	9
	V =	57	54
	S =	40	48
20	P =	32	30
	T =	35	31
	A =	40	37
	Y =	23	27
	H =	13	11
25	E =	51	52
	Q =	26	25
	D =	38	44
	N =	34	35
	K =	32	32
30	C =	20	20
	W =	13	13
	R =	39	41
	G =	51	51
35	deduced Mr = 77253		76699

EXAMPLE V

Northern Blot Analysis of Human and Mouse Transglutaminase mRNA

Hybridization probes suitable for detecting tissue transglutaminase mRNA in tissues were prepared as TG1600 antisense RNA (^{32}P labeled using ^{32}P -UTP). Northern blots were performed according to Thomas, Proc. Natl. Acad. Sci. USA 77:5201 (1980), incorporated herein by reference. Fig. 4 shows the Northern blot analysis of RNA from human umbilical vein

endothelial cells probed with radiolabeled cDNA prepared from the insert in clone HTG-1. A single band at approximately 3.5 kilobases was detected. Fig. 5 shows the results of Northern analysis of RNA's prepared from several mouse tissues (liver, spleen, kidney, testis, heart, lungs, thymus, and brain). Minimal levels of transglutaminase mRNA were detected in thymus and in brain tissues. The levels of this RNA were higher in liver, spleen and testis and were highest in the kidney, lung and heart.

EXAMPLE VI

Expression of Tissue Transglutaminase in Eukaryotic Cells

A cDNA clone for human endothelial cell tissue transglutaminase (clone HTG1) was cloned into the Eco RI site of the eukaryotic expression vector pSG5 (Stratagene). This 3257 bp insert contained 138 nucleotides of 5'-untranslated sequence, the coding region of the enzyme and 1058 bp of 3'-untranslated sequence.

The human tissue transglutaminase expression plasmid was transiently transfected into COS-1 cells using a DEAE-Dextran mediated transfection protocol. Cells were cultured in DMEM containing 10% fetal calf serum (FCS). After 48 and 72 hours cells were washed, scraped and homogenized and the transglutaminase activity was measured as the calcium-dependent covalent incorporation of radiolabeled putrescine into N,N-dimethylcasein (essentially as described by Murtagh et al., J. Biol. Chem. 261:614-621 (1986)). In control COS-1 cells transglutaminase activity was 5.6 fmols/min/mg. In the cells transfected with the transglutaminase expression vector the transglutaminase activity was 270 fmols/min/mg.

BALB/c 3T3 cells were co-transfected (via the CaPO_4 procedure) with the transglutaminase expression vector and an SV-neo containing plasmid (obtained from Clontech). The cells were grown for 48 hours in DMEM containing 10% FCS and 10% Serum Plus (Hazelton Biologics, Inc., Lexena, KS). The cells

were washed and the medium was replaced with DMEM containing 10% FCS, 10% Serum Plus and 400 μ g/ml G-418. G-418 resistant cells were cloned. Individual clones of transfected 3T3 cells were grown to confluency. The cells were then lysed and expression of tissue transglutaminase was measured by Western blot (U.S. Pat. No. 4,452,901; Towbin et al., Proc. Natl. Acad. Sci. USA 76:4350-4358 (1979)) using a polyclonal antibody to guinea pig liver tissue transglutaminase and by enzymatic assay (using the assay described above). Three clones of 3T3 cells (clones 13, 15, and 19) were isolated and characterized in detail. Western blot of the three transfected clones showed a prominent 80,000 kD band of immunoreactivity in the three cell lines, with the abundance in the order cl 15 >> cl 13 > cl 19. No immunoreactive tissue transglutaminase was detected in the non-transfected 3T3 cell extracts. Enzymatic assay of homogenates of the transfected cells showed activities as follows:

	Control 3T3	< 0.17	pmol/min/mg
20	Clone 19	.051	"
	Clone 13	.188	"
	Clone 15	.775	"

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Davies, Peter JA
Stein, Joseph P
- (ii) TITLE OF INVENTION: CLONING AND EXPRESSION OF TISSUE
TRANSGLUTAMINASE
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Steven W. Parmelee
 - (B) STREET: One Market Plaza, Steuart Tower, Suite
2000
 - (C) CITY: San Francisco
 - (D) STATE: California
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 94105
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.24
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/635,756
 - (B) FILING DATE: 04-JAN-1991
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Parmelee, Steven W.
 - (B) REGISTRATION NUMBER: 31,990
 - (C) REFERENCE/DOCKET NUMBER: 13952-7
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (415) 543-9600
 - (B) TELEFAX: (415) 543-5043

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3257 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (D) DEVELOPMENTAL STAGE: Adult
- (F) TISSUE TYPE: Umbilical vein
- (G) CELL TYPE: Endothelial
- (H) CELL LINE: HUVEC

(vii) IMMEDIATE SOURCE:

- (B) CLONE: hTG-1

(viii) POSITION IN GENOME:

- (C) UNITS: bp

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 136..2199
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AACAGGCGTG ACGCCAGTTC TAAACTTGAA ACAAACAAA ACTTCAAAGT ACACCAAAT      60
AGAACCTCCT TAAAGCATAA ATCTCACGGA GGGTCTCGGC CGCCAGTGGA AGGAGCCACC    120
GCCCCGCCCC CGACC ATG GCC GAG GAG CTG GTC TTA GAG AGG TGT GAT CTG      171
      Met Ala Glu Glu Leu Val Leu Glu Arg Cys Asp Leu
              1                      5                      10

GAG CTG GAG ACC AAT GGC CGA GAC CAC CAC ACG GCC GAC CTG TGC CGG      219
Glu Leu Glu Thr Asn Gly Arg Asp His His Thr Ala Asp Leu Cys Arg
              15                      20                      25

GAG AAG CTG GTG GTG CGA CGG GGC CAG CCC TTC TGG CTG ACC CTG CAC      267
Glu Lys Leu Val Val Arg Arg Gly Gln Pro Phe Trp Leu Thr Leu His
              30                      35                      40

TTT GAG GGC CGC AAC TAC GAG GCC AGT GTA GAC AGT CTC ACC TTC AGT      315
Phe Glu Gly Arg Asn Tyr Glu Ala Ser Val Asp Ser Leu Thr Phe Ser
              45                      50                      55

GTC GTG ACC GGC CCA GCC CCT AGC CAG GAG GCC GGG ACC AAG GCC CGT      363
Val Val Thr Gly Pro Ala Pro Ser Gln Glu Ala Gly Thr Lys Ala Arg
              65                      70                      75

TTT CCA CTA AGA GAT GCT GTG GAG GAG GGT GAC TGG ACA GCC ACC GTG      411
Phe Pro Leu Arg Asp Ala Val Glu Glu Gly Asp Trp Thr Ala Thr Val
              80                      85                      90

GTG GAC CAG CAA GAC TGC ACC CTC TCG CTG CAG CTC ACC ACC CCG GCC      459
Val Asp Gln Gln Asp Cys Thr Leu Ser Leu Gln Leu Thr Thr Pro Ala
              95                      100                      105

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AAC Asn	GCC Ala 110	CCC Pro	ATC Ile	GGC Gly	CTG Leu	TAT Tyr 115	CGC Arg	CTC Leu	AGC Ser	CTG Leu	GAG Glu 120	GCC Ala	TCC Ser	ACT Thr	GGC Gly	507
TAC Tyr 125	CAG Gln	GGA Gly	TCC Ser	AGC Ser	TTT Phe 130	GTG Val	CTG Leu	GGC Gly	CAC His	TTC Phe 135	ATT Ile	TTG Leu	CTC Leu	TTC Phe	AAC Asn 140	555
GCC Ala	TGG Trp	TGC Cys	CCA Pro	GCG Ala 145	GAT Asp	GCT Ala	GTG Val	TAC Tyr	CTG Leu 150	GAC Asp	TCG Ser	GAA Glu	GAG Glu	GAG Glu	CGG Arg 155	603
CAG Gln	GAG Glu	TAT Tyr 160	GTC Val	CTC Leu	ACC Thr	CAG Gln	CAG Gln	GGC Gly 165	TTT Phe	ATC Ile	TAC Tyr	CAG Gln	GGC Gly 170	TCG Ser	GCC Ala	651
AAG Lys	TTC Phe	ATC Ile 175	AAG Lys	AAC Asn	ATA Ile	CCT Pro	TGG Trp 180	AAT Asn	TTT Phe	GGG Gly	CAG Gln	TTT Phe 185	CAA Gln	GAT Asp	GGG Gly	699
ATC Ile 190	CTA Leu	GAC Asp	ATC Ile	TGC Cys	CTG Leu	ATC Ile 195	CTT Leu	CTA Leu	GAT Asp	GTC Val	AAC Asn 200	CCC Pro	AAG Lys	TTC Phe	CTG Leu	747
AAG Lys 205	AAC Asn	GCC Ala	GGC Gly	CGT Arg	GAC Asp 210	TGC Cys	TCC Ser	CGG Arg	CGC Arg	AGC Ser 215	AGC Ser	CCC Pro	GTC Val	TAC Tyr	GTG Val 220	795
GGC Gly	CGG Arg	GTG Val	GGT Gly	AGT Ser 225	GGC Gly	ATG Met	GTC Val	AAC Asn	TGC Cys 230	AAC Asn	GAT Asp	GAC Asp	CAG Gln	GGT Gly 235	GTG Val	843
CTG Leu	CTG Leu	GGA Gly 240	CGC Arg	TGG Trp	GAC Asp	AAC Asn	AAC Asn	TAC Tyr 245	GGG Gly	GAC Asp	GGC Gly	GTC Val	AGC Ser 250	CCC Pro	ATG Met	891
TCC Ser	TGG Trp 255	ATC Ile	GGC Gly	AGC Ser	GTG Val	GAC Asp	ATC Ile 260	CTG Leu	CGG Arg	CGC Arg	TGG Trp	AAG Lys 265	AAC Asn	CAC His	GGC Gly	939
TGC Cys 270	CAG Gln	CGC Arg	GTC Val	AAG Lys	TAT Tyr	GGC Gly 275	CAG Gln	TGC Cys	TGG Trp	GTC Val	TTC Phe 280	GCC Ala	GCC Ala	GTG Val	GCC Ala	987
TGC Cys 285	ACA Thr	GTG Val	CTG Leu	AGG Arg	TGC Cys 290	CTA Leu	GGC Gly	ATC Ile	CCT Pro	ACC Thr 295	CGC Arg	GTC Val	GTG Val	ACC Thr	AAC Asn 300	1035
TAC Tyr	AAC Asn	TCG Ser	GCC Ala	CAT His 305	GAC Asp	CAG Gln	AAC Asn	AGC Ser	AAC Asn 310	CTT Leu	CTC Leu	ATC Ile	GAG Glu	TAC Tyr 315	TTC Phe	1083
CGC Arg	AAT Asn	GAG Glu	TTT Phe	GGG Gly	GAG Glu	ATC Ile	CAG Gln	GGT Gly	GAC Asp	AAG Lys	AGC Ser	GAG Glu	ATG Met	ATC Ile	TGG Trp	1131

25

320						325						330						
AAC	TTC	CAC	TGC	TGG	GTG	GAG	TCG	TGG	ATG	ACC	AGG	CCG	GAC	CTG	CAG			1179
Asn	Phe	His	Cys	Trp	Val	Glu	Ser	Trp	Met	Thr	Arg	Pro	Asp	Leu	Gln			
		335					340					345						
CCG	GGG	TAC	GAG	GGC	TGG	CAG	GCC	CTG	GAC	CCA	ACG	CCC	CAG	GAG	AAG			1227
Pro	Gly	Tyr	Glu	Gly	Trp	Gln	Ala	Leu	Asp	Pro	Thr	Pro	Gln	Glu	Lys			
	350					355					360							
AGC	GAA	GGA	ACG	TAC	TGC	TGT	GGC	CCA	GTT	CCA	GTT	CGT	GCC	ATC	AAG			1275
Ser	Glu	Gly	Thr	Tyr	Cys	Cys	Gly	Pro	Val	Pro	Val	Arg	Ala	Ile	Lys			
	365				370					375					380			
GAG	GGC	GAC	CTG	AGC	ACC	AAG	TAC	GAT	GCG	CCC	TTT	GTC	TTT	GCG	GAG			1323
Glu	Gly	Asp	Leu	Ser	Thr	Lys	Tyr	Asp	Ala	Pro	Phe	Val	Phe	Ala	Glu			
				385					390					395				
GTC	AAT	GCC	GAC	GTG	GTA	GAC	TGG	ATC	CAG	CAG	GAC	GAT	GGG	TCT	GTG			1371
Val	Asn	Ala	Asp	Val	Val	Asp	Trp	Ile	Gln	Gln	Asp	Asp	Gly	Ser	Val			
			400					405					410					
CAC	AAA	TCC	ATC	AAC	CGT	TCC	CTG	ATC	GTT	GGG	CTG	AAG	ATC	AGC	ACT			1419
His	Lys	Ser	Ile	Asn	Arg	Ser	Leu	Ile	Val	Gly	Leu	Lys	Ile	Ser	Thr			
		415					420					425						
AAG	AGC	GTG	GGC	CGA	GAC	GAG	CGG	GAG	GAT	ATC	ACC	CAC	ACC	TAC	AAA			1467
Lys	Ser	Val	Gly	Arg	Asp	Glu	Arg	Glu	Asp	Ile	Thr	His	Thr	Tyr	Lys			
	430					435					440							
TAC	CCA	GAG	GGG	TCC	TCA	GAG	GAG	AGG	GAG	GCC	TTC	ACA	AGG	GCG	AAC			1515
Tyr	Pro	Glu	Gly	Ser	Ser	Glu	Glu	Arg	Glu	Ala	Phe	Thr	Arg	Ala	Asn			
	445				450					455					460			
CAC	CTG	AAC	AAA	CTG	GCC	GAG	AAG	GAG	GAG	ACA	GGG	ATG	GCC	ATG	CGG			1563
His	Leu	Asn	Lys	Leu	Ala	Glu	Lys	Glu	Glu	Thr	Gly	Met	Ala	Met	Arg			
				465				470						475				
ATC	CGT	GTG	GGC	CAG	AGC	ATG	AAC	ATG	GGC	AGT	GAC	TTT	GAC	GTC	TTT			1611
Ile	Arg	Val	Gly	Gln	Ser	Met	Asn	Met	Gly	Ser	Asp	Phe	Asp	Val	Phe			
			480					485					490					
GCC	CAC	ATC	ACC	AAC	AAC	ACC	GCT	GAG	GAG	TAC	GTC	TGC	CGC	CTC	CTG			1659
Ala	His	Ile	Thr	Asn	Asn	Thr	Ala	Glu	Glu	Tyr	Val	Cys	Arg	Leu	Leu			
		495					500					505						
CTC	TGT	GCC	CGC	ACC	GTC	AGC	TAC	AAT	GGG	ATC	TTG	GGG	CCC	GAG	TGT			1707
Leu	Cys	Ala	Arg	Thr	Val	Ser	Tyr	Asn	Gly	Ile	Leu	Gly	Pro	Glu	Cys			
	510					515					520							
GGC	ACC	AAG	TAC	CTG	CTC	AAC	CTA	ACC	CTG	GAG	CCT	TTC	TCT	GAG	AAG			1755
Gly	Thr	Lys	Tyr	Leu	Leu	Asn	Leu	Thr	Leu	Glu	Pro	Phe	Ser	Glu	Lys			
	525				530					535					540			
AGC	GTT	CCT	CTT	TGC	ATC	CTC	TAT	GAG	AAA	TAC	CGT	GAC	TGC	CTT	ACG			1803
Ser	Val	Pro	Leu	Cys	Ile	Leu	Tyr	Glu	Lys	Tyr	Arg	Asp	Cys	Leu	Thr			

26

545

550

555

GAG TCC AAC CTC ATC AAG GTG CGG GCC CTC CTC GTG GAG CCA GTT ATC	1851
Glu Ser Asn Leu Ile Lys Val Arg Ala Leu Leu Val Glu Pro Val Ile	
560 565 570	
AAC AGC TAC CTG CTG GCT GAG AGG GAC CTC TAC CTG GAG AAT CCA GAA	1899
Asn Ser Tyr Leu Leu Ala Glu Arg Asp Leu Tyr Leu Glu Asn Pro Glu	
575 580 585	
ATC AAG ATC CGG ATC CTT GGG GAG CCC AAG CAG AAA CGC AAG CTG GTG	1947
Ile Lys Ile Arg Ile Leu Gly Glu Pro Lys Gln Lys Arg Lys Leu Val	
590 595 600	
GCT GAG GTG TCC CTG CAG AAC CCG CTC CCT GTG GCC CTG GAA GGC TGC	1995
Ala Glu Val Ser Leu Gln Asn Pro Leu Pro Val Ala Leu Glu Gly Cys	
605 610 615 620	
ACC TTC ACT GTG GAG GGG GCC GGC CTG ACT GAG GAG CAG AAG ACG GTG	2043
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625 630 635	
GAG ATC CCA GAC CCC GTG GAG GCA GGG GAG GAA GTT AAG GTG AGA ATG	2091
Glu Ile Pro Asp Pro Val Glu Ala Gly Glu Glu Val Lys Val Arg Met	
640 645 650	
GAC CTC GTG CCG CTC CAC ATG GGC CTC CAC AAG CTG GTG GTG AAC TTC	2139
Asp Leu Val Pro Leu His Met Gly Leu His Lys Leu Val Val Asn Phe	
655 660 665	
GAG AGC GAC AAG CTG AAG GCT GTG AAG GGC TTC CGG AAT GTC ATC ATT	2187
Glu Ser Asp Lys Leu Lys Ala Val Lys Gly Phe Arg Asn Val Ile Ile	
670 675 680	
GGC CCC GCC TAA GGGACCCCTG CTCCCAGCCT GCTGAGAGCC CCCACCTTGA	2239
Gly Pro Ala	
685	
TCCCAATCCT TATCCCAAGC TAGTGAGCAA AATATGCCCC TTATTGGGCC CCAGACCCCA	2299
GGGCAGGGTG GGCAGCCTAT GGGGGCTCTC GGAAATGGAA TGTGCCCCCTC GCCCATCTCA	2359
GCCTCCTGAG CCTGTGGGTC CCCACTCACC CCCTTTGCTG TGAGGAATGC TCTGTGCCAG	2419
AAACAGTGGG AGCCCTGACC TGTGCTGACT GGGGCTGGGG TGAGAGAGGA AAGACCTACA	2479
TTCCCTCTCC TGCCCAGATG CCCTTTGGAA AGCCATTGAC CACCCACCAT ATTGTTTGAT	2539
CTACTTCATA GCTCCTTGGA GCAGGCAAAA AAGGGACAGC ATGCCCTTGG CTGGATCAGG	2599
AATCCAGCTC CCTAGACTGC ATCCCGTACC TCTTCCCATG ACTGCACCCA GCTCCAGGGG	2659
CCCTTGGGAC ACCCAGAGCT GGGTGGGGAC AGTGATAGGC CCAAGGTCCC CTCCACATCC	2719
CAGCAGCCCA AGCTTAATAG CCCTCCCCCT CAACCTCACC ATTGTGAAGC ACCTACTATG	2779

TGCTGGGTGC CTCCACACT TGCTGGGGCT CACGGGGCCT CCAACCCATT TAATCACCAT 2839
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 AGCCCCCTCC ACACCAGTGG CCTCGTGGTT ATAAGCAAGG CTGGGTAATG TGAAGGCCCA 2959
 AGAGCAGAGT CTGGGCCTCT GACTCTGAGT CCACTGCTCC ATTTATAACC CCAGCCTGAC 3019
 CTGAGACTGT CGCAGAGGCT GTCTGGGGCC TTTATCAAAA AAAGACTCAG CCAAGACAAG 3079
 GAGGTAGAGA GGGGACTGGG GGAAGTGGGAG TCAGAGCCCT GGCTGGGTTC AGGTCCCACG 3139
 TCTGGCCAGG CACTGCCTTC TCCTCTCTGG GCCTTTGTTT CCTTGTTGGT CAGAGGAGTG 3199
 ATTGAACCTG CTCATCTCCA AGGATCCTCT CCACTCCATG TTTGCAATAC ACAATTCC 3257

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 687 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ala	Glu	Glu	Leu	Val	Leu	Glu	Arg	Cys	Asp	Leu	Glu	Leu	Glu	Thr	1	5	10	15
Asn	Gly	Arg	Asp	His	His	Thr	Ala	Asp	Leu	Cys	Arg	Glu	Lys	Leu	Val	20	25	30	
Val	Arg	Arg	Gly	Gln	Pro	Phe	Trp	Leu	Thr	Leu	His	Phe	Glu	Gly	Arg	35	40	45	
Asn	Tyr	Glu	Ala	Ser	Val	Asp	Ser	Leu	Thr	Phe	Ser	Val	Val	Thr	Gly	50	55	60	
Pro	Ala	Pro	Ser	Gln	Glu	Ala	Gly	Thr	Lys	Ala	Arg	Phe	Pro	Leu	Arg	65	70	75	80
Asp	Ala	Val	Glu	Glu	Gly	Asp	Trp	Thr	Ala	Thr	Val	Val	Asp	Gln	Gln	85	90	95	
Asp	Cys	Thr	Leu	Ser	Leu	Gln	Leu	Thr	Thr	Pro	Ala	Asn	Ala	Pro	Ile	100	105	110	
Gly	Leu	Tyr	Arg	Leu	Ser	Leu	Glu	Ala	Ser	Thr	Gly	Tyr	Gln	Gly	Ser	115	120	125	
Ser	Phe	Val	Leu	Gly	His	Phe	Ile	Leu	Leu	Phe	Asn	Ala	Trp	Cys	Pro	130	135	140	

Ala 145	Asp	Ala	Val	Tyr	Leu 150	Asp	Ser	Glu	Glu	Glu 155	Arg	Gln	Glu	Tyr	Val 160
Leu	Thr	Gln	Gln	Gly 165	Phe	Ile	Tyr	Gln	Gly 170	Ser	Ala	Lys	Phe	Ile 175	Lys
Asn	Ile	Pro	Trp 180	Asn	Phe	Gly	Gln	Phe 185	Gln	Asp	Gly	Ile	Leu 190	Asp	Ile
Cys	Leu	Ile 195	Leu	Leu	Asp	Val	Asn 200	Pro	Lys	Phe	Leu	Lys 205	Asn	Ala	Gly
Arg	Asp 210	Cys	Ser	Arg	Arg	Ser 215	Ser	Pro	Val	Tyr	Val 220	Gly	Arg	Val	Gly
Ser 225	Gly	Met	Val	Asn	Cys 230	Asn	Asp	Asp	Gln	Gly 235	Val	Leu	Leu	Gly	Arg 240
Trp	Asp	Asn	Asn	Tyr 245	Gly	Asp	Gly	Val	Ser 250	Pro	Met	Ser	Trp	Ile 255	Gly
Ser	Val	Asp	Ile 260	Leu	Arg	Arg	Trp	Lys 265	Asn	His	Gly	Cys	Gln 270	Arg	Val
Lys	Tyr	Gly 275	Gln	Cys	Trp	Val	Phe 280	Ala	Ala	Val	Ala	Cys 285	Thr	Val	Leu
Arg	Cys 290	Leu	Gly	Ile	Pro	Thr 295	Arg	Val	Val	Thr	Asn 300	Tyr	Asn	Ser	Ala
His 305	Asp	Gln	Asn	Ser	Asn 310	Leu	Leu	Ile	Glu	Tyr 315	Phe	Arg	Asn	Glu	Phe 320
Gly	Glu	Ile	Gln	Gly 325	Asp	Lys	Ser	Glu	Met 330	Ile	Trp	Asn	Phe	His 335	Cys
Trp	Val	Glu	Ser 340	Trp	Met	Thr	Arg	Pro 345	Asp	Leu	Gln	Pro	Gly 350	Tyr	Glu
Gly	Trp	Gln 355	Ala	Leu	Asp	Pro	Thr 360	Pro	Gln	Glu	Lys	Ser 365	Glu	Gly	Thr
Tyr	Cys 370	Cys	Gly	Pro	Val	Pro 375	Val	Arg	Ala	Ile	Lys 380	Glu	Gly	Asp	Leu
Ser 385	Thr	Lys	Tyr	Asp	Ala 390	Pro	Phe	Val	Phe	Ala 395	Glu	Val	Asn	Ala	Asp 400
Val	Val	Asp	Trp	Ile 405	Gln	Gln	Asp	Asp	Gly 410	Ser	Val	His	Lys	Ser 415	Ile
Asn	Arg	Ser	Leu 420	Ile	Val	Gly	Leu	Lys 425	Ile	Ser	Thr	Lys	Ser 430	Val	Gly
Arg	Asp	Glu 435	Arg	Glu	Asp	Ile	Thr	His	Thr	Tyr	Lys	Tyr 445	Pro	Glu	Gly

Ser Ser Glu Glu Arg Glu Ala Phe Thr Arg Ala Asn His Leu Asn Lys
 450 455 460
 Leu Ala Glu Lys Glu Glu Thr Gly Met Ala Met Arg Ile Arg Val Gly
 465 470 475 480
 Gln Ser Met Asn Met Gly Ser Asp Phe Asp Val Phe Ala His Ile Thr
 485 490 495
 Asn Asn Thr Ala Glu Glu Tyr Val Cys Arg Leu Leu Leu Cys Ala Arg
 500 505 510
 Thr Val Ser Tyr Asn Gly Ile Leu Gly Pro Glu Cys Gly Thr Lys Tyr
 515 520 525
 Leu Leu Asn Leu Thr Leu Glu Pro Phe Ser Glu Lys Ser Val Pro Leu
 530 535 540
 Cys Ile Leu Tyr Glu Lys Tyr Arg Asp Cys Leu Thr Glu Ser Asn Leu
 545 550 555 560
 Ile Lys Val Arg Ala Leu Leu Val Glu Pro Val Ile Asn Ser Tyr Leu
 565 570 575
 Leu Ala Glu Arg Asp Leu Tyr Leu Glu Asn Pro Glu Ile Lys Ile Arg
 580 585 590
 Ile Leu Gly Glu Pro Lys Gln Lys Arg Lys Leu Val Ala Glu Val Ser
 595 600 605
 Leu Gln Asn Pro Leu Pro Val Ala Leu Glu Gly Cys Thr Phe Thr Val
 610 615 620
 Glu Gly Ala Gly Leu Thr Glu Glu Gln Lys Thr Val Glu Ile Pro Asp
 625 630 635 640
 Pro Val Glu Ala Gly Glu Glu Val Lys Val Arg Met Asp Leu Val Pro
 645 650 655
 Leu His Met Gly Leu His Lys Leu Val Val Asn Phe Glu Ser Asp Lys
 660 665 670
 Leu Lys Ala Val Lys Gly Phe Arg Asn Val Ile Ile Gly Pro Ala
 675 680 685

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3486 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
- (D) DEVELOPMENTAL STAGE: Adult
- (F) TISSUE TYPE: peritoneal
- (G) CELL TYPE: macrophage

(viii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 30..2087
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGTCTGAGCT	GTGCGCCGCTA	GCCTGGCCCC	ATG	GCA	GAG	GAG	CTG	CTC	CTA	GAG	53
			Met	Ala	Glu	Glu	Leu	Leu	Leu	Glu	
			1				5				
AGG	TGT	GAT	CTG	GAG	ATT	CAG	GCC	AAT	GGC	CGT	101
Arg	Cys	Asp	Leu	Glu	Ile	Gln	Ala	Asn	Gly	Arg	
	10					15				20	
GAC	CTA	TGC	CAA	GAG	AAA	CTG	CTG	GTG	CGT	CGT	149
Asp	Leu	Cys	Gln	Glu	Lys	Leu	Leu	Val	Arg	Arg	
25					30				35		
GAC	CTA	TGC	CAA	GAG	AAA	CTG	CTG	GTG	CGT	CGT	197
Asp	Leu	Cys	Gln	Glu	Lys	Leu	Leu	Val	Arg	Arg	
25					30				35		
CTG	ACT	CTG	TAC	TTC	GAG	GGC	CGT	GGC	TAC	GAG	245
Leu	Thr	Leu	Tyr	Phe	Glu	Gly	Arg	Gly	Tyr	Glu	
			45					50			
CTC	ACG	TTC	GGT	GCT	GTG	ACC	GGC	CCA	GAT	CCC	293
Leu	Thr	Phe	Gly	Ala	Val	Thr	Gly	Pro	Asp	Pro	
			60					65			
ACC	AAG	GCC	CGC	TTT	TCA	CTG	TCT	GAC	AAT	GTG	341
Thr	Lys	Ala	Arg	Phe	Ser	Leu	Ser	Asp	Asn	Val	
	75						80				
TCA	GCC	TCA	CTG	GTG	GAC	CAG	CAG	GAC	AAT	GTC	389
Ser	Ala	Ser	Leu	Val	Asp	Gln	Gln	Asp	Asn	Val	
	90					95				100	
TGC	ACC	CCA	GCC	AAT	GCT	CCT	ATT	GGC	CTG	TAC	437
Cys	Thr	Pro	Ala	Asn	Ala	Pro	Ile	Gly	Leu	Tyr	
105					110				115		
GCT	TCT	ACT	GGC	TAC	CAG	GGC	TCC	AGC	TTT	GTG	
Ala	Ser	Thr	Gly	Tyr	Gln	Gly	Ser	Ser	Phe	Val	

31

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TTG	CTC	TAC	AAT	GCC	TGG	TGC	CCA	GCC	GAT	GAT	GTG	TAC	CTA	GAC	TCA	485																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	

345	350	355	360	
CCA CAG GAG AAG AGC GAA GGG ACA TAC TGT TGT GGC CCA GTC TCA GTG Pro Gln Glu Lys Ser Glu Gly Thr Tyr Cys Cys Gly Pro Val Ser Val 365 370 375				1157
CGA GCC ATC AAG GAG GGA GAC CTG AGT ACC AAG TAT GAT GCA CCC TTC Arg Ala Ile Lys Glu Gly Asp Leu Ser Thr Lys Tyr Asp Ala Pro Phe 380 385 390				1205
GTG TTT GCC GAG GTC AAC GCT GAT GTG GTG GAC TGG ATC CGG CAG GAC Val Phe Ala Glu Val Asn Ala Asp Val Val Asp Trp Ile Arg Gln Asp 395 400 405				1253
GAA GGG TCT GTG CTC AAA TGG ATG AAC CGT TCC TTG GTC GTG GGG CAG Glu Gly Ser Val Leu Lys Trp Met Asn Arg Ser Leu Val Val Gly Gln 410 415 420				1301
AAG ATC AGC ACT AAG AGT GTG GGC CGT GAT GAC CGG GAG GAC ATC ACC Lys Ile Ser Thr Lys Ser Val Gly Arg Asp Asp Arg Glu Asp Ile Thr 425 430 435 440				1349
CAT ACA TAC AAG TAC CCA GAG GGG TCA CCC GAG GAG AGG GAA GTC TTC His Thr Tyr Lys Tyr Pro Glu Gly Ser Pro Glu Glu Arg Glu Val Phe 445 450 455				1397
ACC AAG GCC AAC CAC CTG AAC AAA CTG GAC GAG AAA GAG GGG ACA GGG Thr Lys Ala Asn His Leu Asn Lys Leu Asp Glu Lys Glu Gly Thr Gly 460 465 470				1445
ATG GCC ATG CGC ATC CGA GTG GGG CAG TAT GAG CAT GGC AAC GAC TTC Met Ala Met Arg Ile Arg Val Gly Gln Tyr Glu His Gly Asn Asp Phe 475 480 485				1493
GAC GTG TTT GCC CAC ATC GGC AAC GAC ACC TCG GAG ACT CGA GAG TGT Asp Val Phe Ala His Ile Gly Asn Asp Thr Ser Glu Thr Arg Glu Cys 490 495 500				1541
CGT CTC CTG CTC TGT GCC CGC ACT GTC AGC TAC AAC GGG GTG CTG GGG Arg Leu Leu Leu Cys Ala Arg Thr Val Ser Tyr Asn Gly Val Leu Gly 505 510 515 520				1589
CCC GAG TGT GGC ACT GAG GAC ATC AAC CTG ACC CTG GAT CCC TAC TCT Pro Glu Cys Gly Thr Glu Asp Ile Asn Leu Thr Leu Asp Pro Tyr Ser 525 530 535				1637
GAG AAC AGC ATC CCA CTT CGA ATC CTC TAC GAG AAG TAC AGC GGG TGC Glu Asn Ser Ile Pro Leu Arg Ile Leu Tyr Glu Lys Tyr Ser Gly Cys 540 545 550				1685
CGT ACA GAG TCA AAC CTC ATC AAG GTG CGG GGC CTT CTC ATC GAA CCA Arg Thr Glu Ser Asn Leu Ile Lys Val Arg Gly Leu Leu Ile Glu Pro 555 560 565				1733
GCT GCC AAC AGC TAC CTG CTG GCT GAG AGA GAT CTC TAC GTG GAG AAT				1781

Ala	Ala	Asn	Ser	Tyr	Leu	Leu	Ala	Glu	Arg	Asp	Leu	Tyr	Val	Glu	Asn	
570						575					580					
CCC	GAA	ATC	AAG	ATC	CGG	GTT	TTG	GGA	GAA	CCC	AAG	CAA	AAC	CGC	AAA	1829
Pro	Glu	Ile	Lys	Ile	Arg	Val	Leu	Gly	Glu	Pro	Lys	Gln	Asn	Arg	Lys	
585					590					595					600	
CTG	GTG	GCT	GAG	GTG	TCC	CTG	AAG	AAC	CCA	CTT	TCC	GAT	CCC	CTG	TAT	1877
Leu	Val	Ala	Glu	Val	Ser	Leu	Lys	Asn	Pro	Leu	Ser	Asp	Pro	Leu	Tyr	
				605					610					615		
GAC	TGC	ATC	TTC	ACT	GTG	GAG	GGG	GCT	GGC	CTG	ACC	AAG	GAG	CAG	AAG	1925
Asp	Cys	Ile	Phe	Thr	Val	Glu	Gly	Ala	Gly	Leu	Thr	Lys	Glu	Gln	Lys	
			620					625					630			
TCT	GTG	GAA	GTC	TCA	GAC	CCG	GTG	CCA	GCG	GGC	GAT	TTG	GTC	AAG	GCA	1973
Ser	Val	Glu	Val	Ser	Asp	Pro	Val	Pro	Ala	Gly	Asp	Leu	Val	Lys	Ala	
		635					640					645				
CGG	GTC	GAC	CTG	TCC	CCG	ACT	GAT	ATT	GGC	CTC	CAC	AAG	CTG	GTG	GTG	2021
Arg	Val	Asp	Leu	Ser	Pro	Thr	Asp	Ile	Gly	Leu	His	Lys	Leu	Val	Val	
	650					655					660					
AAC	TTC	CAG	TGT	GAC	AAG	CTG	AAG	TCG	GTG	AAG	GGT	TAC	CGG	AAT	GTT	2069
Asn	Phe	Gln	Cys	Asp	Lys	Leu	Lys	Ser	Val	Lys	Gly	Tyr	Arg	Asn	Val	
665					670					675					680	
ATC	ATC	GGC	CCG	GCC	TAA	GGG	ACCCCTT	CCC	AGACTCA	ACCCC	ACCAC					2117
Ile	Ile	Gly	Pro	Ala												
				685												
CTGCCA	ACCC	CCATT	CAACC	TGGT	CTTTAT	CCTA	AGATAA	TGAG	CAACTT	CACCCC	ATTC					2177
AGGCT	GACAT	GGCT	GCCTGG	GGCCT	CTTCA	GAAG	ACAGTG	TACTT	CTGGC	CCAAT	CCTGT					2237
TCCT	CTGGAT	CTATTT	CCCC	ATCCT	GTTCC	CTTAGT	GTGC	ACGGA	AGGTC	CTGTG	CCGAC					2297
ACAGT	GGGTA	CCTGT	GGAAA	GGGTA	AGAGG	AGAGC	CATCA	CCAGC	ACTCT	GTATC	TCTGC					2357
ATTG	TTTGAA	CTGT	TCTCTGG	AGCCT	CAGCG	CAAGC	ACAAA	GGGAC	CGTGC	GCATG	GCACC					2417
ATCGA	AGGAA	ACGAT	CCTTG	GAGC	AGGAAC	GCTGT	CGGCA	CCATT	TGCGC	TCCTG	AATGG					2477
AACCA	TATGT	GCATG	GTACT	TAGAT	CCTTAC	GGTAC	ACCAG	CTAGC	GTACA	TCCGT	GTAAC					2537
TTCAG	GTGGT	ACAA	ACTGAG	GCTG	CTGTGC	TGTACT	GGA	CAGTA	GGCAG	GCCAT	CACTT					2597
GCAGG	GCCAG	TGGG	TGGAGC	TGGA	ATACAG	GGAAT	CCATC	TGTG	ACCA	GCTCT	GACCT					2657
GAGCG	GGTCA	GAGAG	GCTAT	CTGGG	GATGA	TGAGC	CTCGC	GGTGG	TAGG	GTGAG	GAGTT					2717
GAGGG	TGGGG	CGGG	GAGGCC	AGAGA	ACTGG	GAGTC	CAGAGC	TTGTG	TTTAA	GCCCC	AAGGA					2777
GAGCT	ACACT	CTATC	CTCCT	CTTCT	GGGCT	TGATC	ATTCT	TCACC	AGGGC	AGTG	CTTAA					2837

CCAGGGGTCT CCTGGCCCTA TACTTCTAAA GCCGTGCTTG CCAGGCTCAG TGCACTTCTG 2897
 GGAAGGAGAC ACTGCACCAG ACCTGTACCC GAGGTGTCCT GCTTCTCACC TATGACTGGG 2957
 TTCCTCAGAT GGCAGTTCCA GGGACTATCC ACAAGCTACT CACACAGTGC CTAGACTAGA 3017
 TTTCACAGAA CGAGCCTTAA ATATCCACGC TGGTCGCTAA TCAGCAGGCC ACACCTCCAC 3077
 CAGCCCCTCC TCCTCAGCCA GCAGCCTCTA GACACAGCAC GTGTCTCCAG AAAAGCGTGG 3137
 AAGCCTGTGT GTGGGTCATC ATCCCAAGTT AGCCCACTCC TACCTCTCTG TGGCTGGCCC 3197
 TCAACCTAGG AAGGCTGGCA GTGGTGGCCG GGTCTCTGGG CGATGAGGTC AGAGTCTCTG 3257
 GATCCCCTGA AATCCCGGAG AAGAGCCTGG GAAGAATCAA ACTGATGCAT TTAACGCGTT 3317
 CTGCTTTACA CAGAGGATCG CACCGTGAGC CGTGCTATCT GTCCTGTCCC CACACGGTTC 3377
 TGTTCCTTTT GGTCTGTGCA AGCTTCAGGT TCCAAGCAGG CAGTAGGCAT GCTTTCAAAG 3437
 CACATGTGAA CACTGAAATA AAGGTCTATT TTTCACATTC ACGGAATTC 3486

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 685 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Glu Glu Leu Leu Leu Glu Arg Cys Asp Leu Glu Ile Gln Ala
 1 5 10 15
 Asn Gly Arg Asp His His Thr Ala Asp Leu Cys Gln Glu Lys Leu Leu
 20 25 30
 Val Arg Arg Gly Gln Arg Phe Arg Leu Thr Leu Tyr Phe Glu Gly Arg
 35 40 45
 Gly Tyr Glu Ala Ser Val Asp Ser Leu Thr Phe Gly Ala Val Thr Gly
 50 55 60
 Pro Asp Pro Ser Glu Glu Ala Gly Thr Lys Ala Arg Phe Ser Leu Ser
 65 70 75 80
 Asp Asn Val Glu Glu Gly Ser Trp Ser Ala Ser Leu Val Asp Gln Gln
 85 90 95
 Asp Asn Val Leu Ser Leu Gln Leu Cys Thr Pro Ala Asn Ala Pro Ile
 100 105 110
 Gly Leu Tyr Arg Leu Ser Leu Glu Ala Ser Thr Gly Tyr Gln Gly Ser

115					120					125					
Ser	Phe	Val	Leu	Gly	His	Phe	Ile	Leu	Leu	Tyr	Asn	Ala	Trp	Cys	Pro
130					135					140					
Ala	Asp	Asp	Val	Tyr	Leu	Asp	Ser	Glu	Glu	Glu	Arg	Arg	Glu	Tyr	Val
145					150					155					160
Leu	Thr	Gln	Gln	Gly	Phe	Ile	Tyr	Gln	Gly	Ser	Val	Lys	Phe	Ile	Lys
				165					170					175	
Ser	Val	Pro	Trp	Asn	Phe	Gly	Gln	Phe	Gln	Asp	Gly	Ile	Leu	Asp	Thr
			180					185					190		
Cys	Leu	Met	Leu	Leu	Asp	Met	Asn	Pro	Lys	Phe	Leu	Lys	Asn	Arg	Ser
		195					200					205			
Arg	Asp	Cys	Ser	Arg	Arg	Ser	Ser	Pro	Ile	Tyr	Val	Gly	Arg	Val	Val
	210					215					220				
Ser	Asp	Met	Val	Asn	Cys	Asn	Asp	Asp	Gln	Gly	Val	Leu	Leu	Gly	Arg
225				230					235						240
Trp	Asp	Asn	Asn	Tyr	Gly	Asp	Gly	Ile	Ser	Pro	Met	Ala	Trp	Ile	Gly
				245					250					255	
Ser	Val	Asp	Ile	Leu	Arg	Arg	Trp	Lys	Glu	His	Gly	Cys	Gln	Gln	Val
			260					265					270		
Lys	Tyr	Gly	Gln	Cys	Trp	Val	Phe	Ala	Ala	Val	Ala	Cys	Thr	Val	Leu
		275					280					285			
Arg	Cys	Leu	Gly	Ile	His	Asn	Arg	Val	Val	Thr	Asn	Tyr	Asn	Ser	Ala
	290					295					300				
His	Asp	Gln	Asn	Ser	Asn	Leu	Leu	Ile	Glu	Tyr	Phe	Arg	Asn	Glu	Phe
305					310				315					320	
Gly	Glu	Leu	Glu	Thr	Asn	Lys	Ser	Glu	Met	Ile	Trp	Asn	Phe	His	Cys
				325					330					335	
Trp	Val	Glu	Ser	Trp	Met	Thr	Arg	Pro	Asp	Leu	Gln	Pro	Gly	Tyr	Glu
			340					345					350		
Gly	Trp	Glu	Ala	Leu	Asp	Pro	Thr	Pro	Gln	Glu	Lys	Ser	Glu	Gly	Thr
		355					360					365			
Tyr	Cys	Cys	Gly	Pro	Val	Ser	Val	Arg	Ala	Ile	Lys	Glu	Gly	Asp	Leu
	370					375					380				
Ser	Thr	Lys	Tyr	Asp	Ala	Pro	Phe	Val	Phe	Ala	Glu	Val	Asn	Ala	Asp
385				390					395						400
Val	Val	Asp	Trp	Ile	Arg	Gln	Asp	Glu	Gly	Ser	Val	Leu	Lys	Trp	Met
				405					410					415	

Asn Arg Ser Leu Val Val Gly Gln Lys Ile Ser Thr Lys Ser Val Gly
 420 425 430
 Arg Asp Asp Arg Glu Asp Ile Thr His Thr Tyr Lys Tyr Pro Glu Gly
 435 440 445
 Ser Pro Glu Glu Arg Glu Val Phe Thr Lys Ala Asn His Leu Asn Lys
 450 455 460
 Leu Asp Glu Lys Glu Gly Thr Gly Met Ala Met Arg Ile Arg Val Gly
 465 470 475 480
 Gln Tyr Glu His Gly Asn Asp Phe Asp Val Phe Ala His Ile Gly Asn
 485 490 495
 Asp Thr Ser Glu Thr Arg Glu Cys Arg Leu Leu Leu Cys Ala Arg Thr
 500 505 510
 Val Ser Tyr Asn Gly Val Leu Gly Pro Glu Cys Gly Thr Glu Asp Ile
 515 520 525
 Asn Leu Thr Leu Asp Pro Tyr Ser Glu Asn Ser Ile Pro Leu Arg Ile
 530 535 540
 Leu Tyr Glu Lys Tyr Ser Gly Cys Arg Thr Glu Ser Asn Leu Ile Lys
 545 550 555 560
 Val Arg Gly Leu Leu Ile Glu Pro Ala Ala Asn Ser Tyr Leu Leu Ala
 565 570 575
 Glu Arg Asp Leu Tyr Val Glu Asn Pro Glu Ile Lys Ile Arg Val Leu
 580 585 590
 Gly Glu Pro Lys Gln Asn Arg Lys Leu Val Ala Glu Val Ser Leu Lys
 595 600 605
 Asn Pro Leu Ser Asp Pro Leu Tyr Asp Cys Ile Phe Thr Val Glu Gly
 610 615 620
 Ala Gly Leu Thr Lys Glu Gln Lys Ser Val Glu Val Ser Asp Pro Val
 625 630 635 640
 Pro Ala Gly Asp Leu Val Lys Ala Arg Val Asp Leu Ser Pro Thr Asp
 645 650 655
 Ile Gly Leu His Lys Leu Val Val Asn Phe Gln Cys Asp Lys Leu Lys
 660 665 670
 Ser Val Lys Gly Tyr Arg Asn Val Ile Ile Gly Pro Ala
 675 680 685

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not
5 limited except as by the appended claims.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide sequence which codes for human tissue transglutaminase.

2. The polynucleotide sequence of claim 1, wherein the transglutaminase is of endothelial cell origin.

3. The polynucleotide molecule of claim 1, wherein the polypeptide encoded thereby catalyzes the Ca^{++} dependent crosslinking of protein-bound glutamine and lysine residues.

4. The polynucleotide of claim 1, wherein the sequence is substantially the human transglutaminase of Fig. 3.

5. The polynucleotide of claim 1, which is a cDNA sequence.

6. An isolated polynucleotide sequence which codes for mouse tissue transglutaminase.

7. The polynucleotide sequence of claim 6, wherein the mouse transglutaminase is of macrophage origin.

8. The polynucleotide of claim 6, wherein the sequence is substantially the murine transglutaminase of Fig. 3.

9. The polynucleotide molecule of claim 6, wherein the polypeptide encoded thereby catalyzes the Ca^{++} dependent crosslinking of protein-bound glutamine and lysine residues.

10. A DNA construct for the expression of human tissue transglutaminase, which comprises the following operably linked elements:

- a transcriptional promoter;
- 5 a DNA sequence encoding a human tissue transglutaminase polypeptide; and
- a transcriptional terminator.

11. The polypeptide which is encoded by the DNA
10 construct of claim 10.

12. The polypeptide of claim 11, which catalyzes Ca^{++} dependent crosslinking of protein-bound glutamine and lysine residues.

13. The polypeptide of claim 12, which has substantially the amino acid sequence of human tissue transglutaminase of Fig. 3.

14. A cultured cell transformed or transfected with the DNA construct of claim 10.

15. The cultured cell of claim 14, which is a eukaryotic cell.

16. The eukaryotic cell of claim 15, which is a yeast cell or mammalian cell.

17. A method for producing human tissue
30 transglutaminase, which comprises cultivating eukaryotic cells transformed or transfected with the DNA construct of claim 11, and isolating the transglutaminase from the cells.

18. The method of claim 17, wherein the
35 transformed eukaryotic cells are yeast cells.

19. A probe which comprises an oligonucleotide of at least about 14 nucleotides capable of specifically hybridizing with a gene which encodes a human or murine tissue transglutaminase polypeptide, wherein said probe is at least
5 85% homologous to a sequence of the human or murine transglutaminase of Fig. 3.

20. The probe of claim 19, which is labeled to provide a detectable signal.

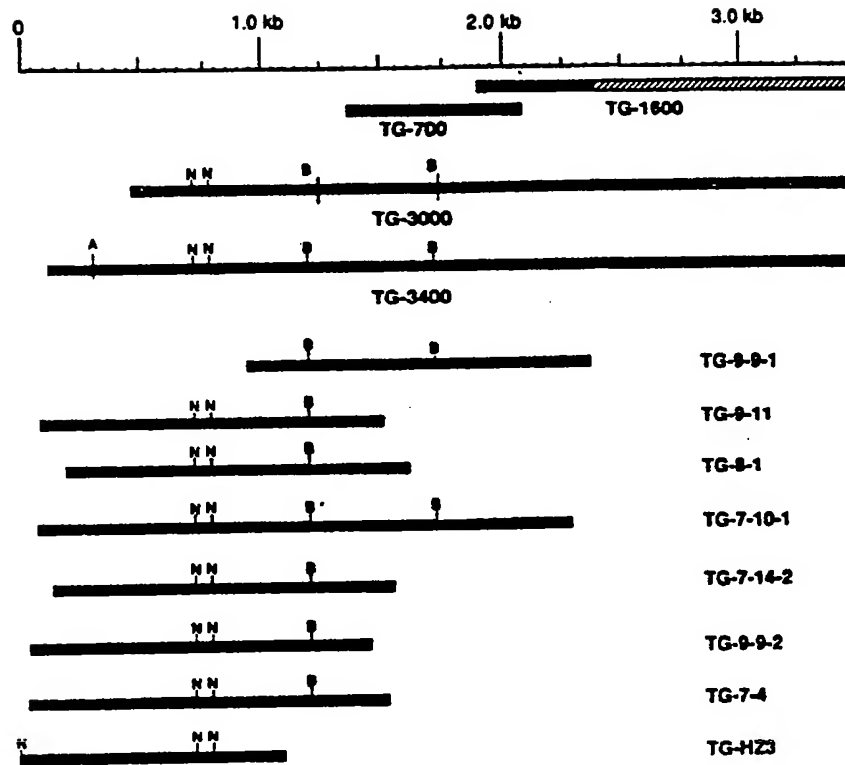


Fig. 1

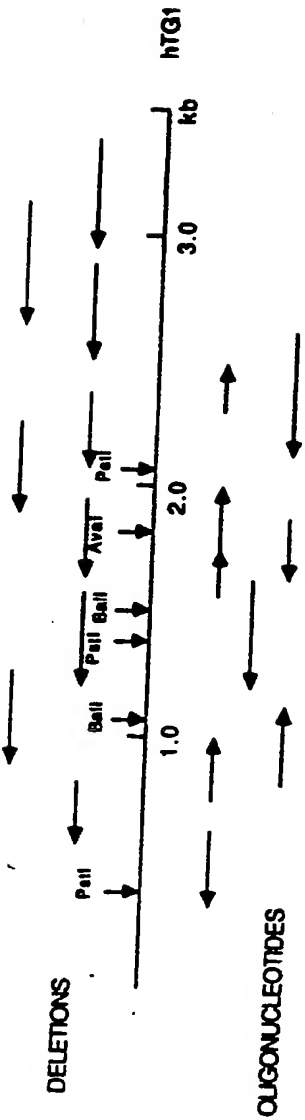


Fig. 2

100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000
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Fig. 3

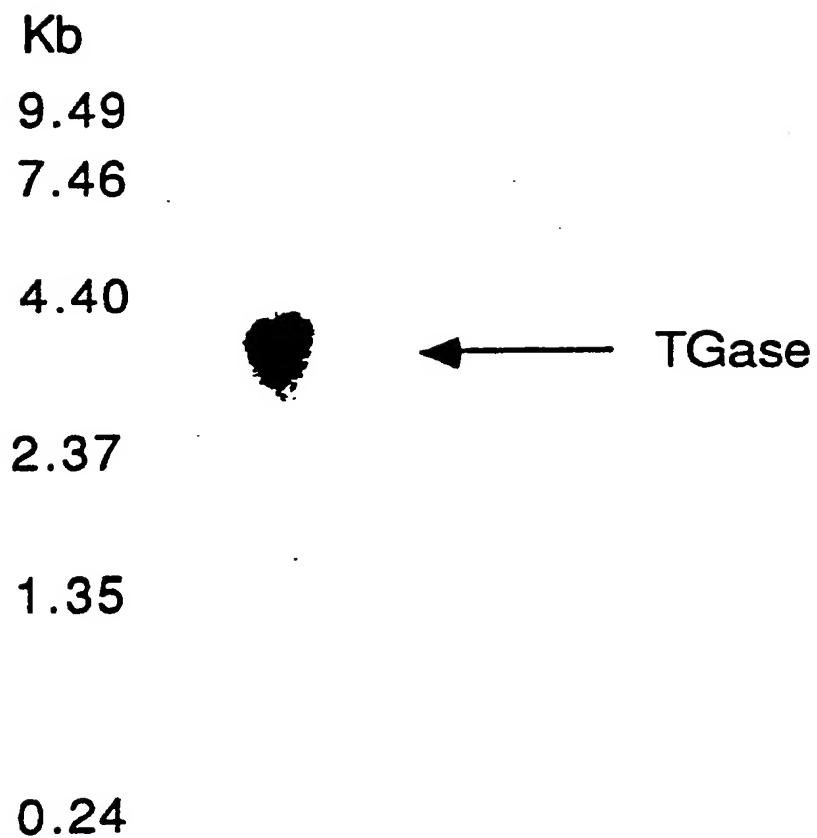


Fig. 4

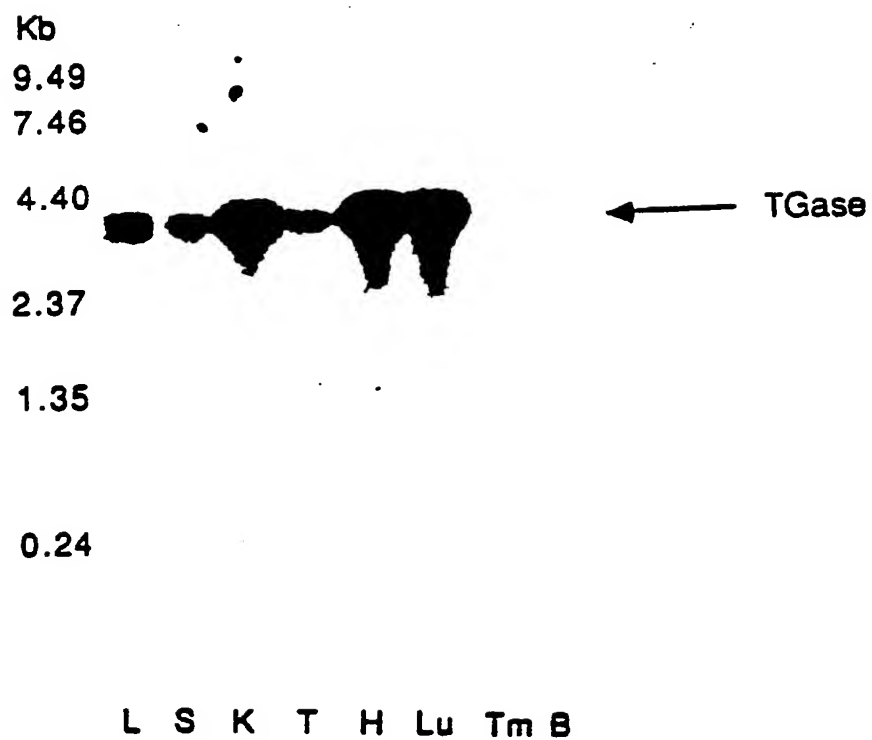


Fig. 5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09784

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5): C12N 9/10, 15/54, 15/62, 15/80, 15/85 U.S.C1.: 435/69.1, 193, 252.3, 320.1; 536/27; 935/8, 14, 68, 70		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S.C1.	435/69.1, 193, 252.3, 320.1; 536/27; 935/8, 14, 68, 70	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸		
APS, STN/Chemical Abstracts and DIALOG/Biosis databases		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
<u>X,O</u> Y	THE JOURNAL OF CELL BIOLOGY, Vol. 109, No. 4 Part 2, issued 9 November 1989, Gentile et al., "Isolation and Characterization of cDNA and Genomic Clones of Human Endothelial Cell Transglutaminases", page 198a, see abstract 1068.	1-5, 19-20 10-17
<u>X,O</u> Y	FASEB JOURNAL, Vol. 3, No. 4, issued 17 February 1989 Saydak et al., "cDNA Cloning of Mouse Macrophage Tissue Transglutaminase", page A1209, see abstract 5708.	6-9, 19-20 10-17
<u>X,O</u> Y	2nd INTERNATIONAL CONFERENCE ON TRANSGLUTAMINASES & PROTEIN CROSS-LINKING REACTIONS, 24-28 June 1990, Gentile et al., "Molecular Cloning and Sequence Analysis of cDNA for a Retinoic Acid-Inducible Tissue Transglutaminase from Human Umbilical Vein Endothelial Cells", see abstract.	1-5, 10, 14-16, 19-20 6-9, 11-13, 15-18
Y	US, A, 4,929,554, GOEDDEL et al., 29 May 1990, see columns 17-21, figures 7-8 and 11-16.	10-17
Y	EP, A, 0,268,772, DAVIE et al., 01 June 1988, see pages 16-20, Figures 5-11.	10-18
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
26 March 1992		20 APR 1992
International Searching Authority		Signature of Authorized Officer
ISA/US		William W. Moore <div style="text-align: right; font-size: 0.8em;">gd</div>